the genetic constitution of the implant itself as well as the relative stages of development of implant and host may also influence this property of autonomy or non-autonomy of the implant.

As a concluding example, we shall mention briefly, experiments made to establish the time in development at which vermilion pigmentation is irreversibly determined, i.e., after which a vermilion eye disc implanted in a wild type host will no longer give wild type pigmentation. By implanting eye discs from *v* pupae of successively older stages in wild type larvae almost ready to pupate (this procedure was used because of technical difficulties in implanting eye discs in pupae), it was shown that the characteristic host-implant influence operates, under these conditions, until very late stages, until about 48 hours after the formation of a puparium (at 25°C.). Shortly after this time, actual pigment can be seen in the eye.

Details of the above-mentioned experiments, as well as a description of the technic, will be published elsewhere.

[1] The work on which this report is based was done at the Institut de Biologie physico-chimique, Paris.

[2] Sturtevant, A. H., *Proc. VI Int. Cong. Genet.*, **1**, 304 (1932).

[3] Bytinski-Salz, H., *Arch. f. Entw.-mech.*, **129**, 356 (1933).

[4] Caspari, E., *Ibid.*, **130**, 353 (1933).

[5] Morgan, T. H., Bridges, C. B., and Sturtevant, A. H., *Bibliog. Genet.*, **2**, 1 (1925).

[6] Ephrussi, Boris, and Beadle, G. W., *Comptes rendus acad. sci.*, **201**, 98 (1935).

[7] Beadle, G. W., and Ephrussi, Boris, *Ibid.*, **201**, 620 (1935).

THE TEMPERATURE-EFFECTIVE PERIOD OF THE SCUTE-1 PHENOTYPE

BY PHILIP TRUMAN IVES*

WILLIAM G. KERCKHOFF LABORATORIES, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA

Communicated November 12, 1935

Introduction.—The recently published papers of Child[1] have shown that for temperatures ranging from 14 to 31°C. there exists a temperature-effective period for the scute-1 phenotype which lies entirely within the latter half of the third larval instar period, when a mass of flies is concerned. For all bristles of the scute pattern, and at all temperatures tested, the effective period occupies the same relative position in the developmental period; and it is apparently restricted to the period between 89.3 and 96.8% of egg-larval development for any individual fly.

The study reported here is likewise on the temperature-effective period

of the scute-1 phenotype, and differs only in the technique used. The temperatures used in this experiment were 25° and 40° to 41°C. Developing larvae cannot usually live through more than five hours at 40° to 41°C.; but three-hour exposures are generally not excessively lethal.

The results of the study are interesting largely because they differ markedly from those of Child. A temperature-effective period appears which, for the ocellar bristles, extends through all the developmental periods tested, from the time of egg laying through the early pupal period. Other bristles considered also show effective periods which lie outside the time of Child's temperature-effective period.

Conditions and Methods.—Before being used in this study, the scute (*sc*) and vermilion carnation (*v car*) stocks were selected for "perfect" scute and normal bristle patterns, respectively, for six generations during the course of a preliminary experiment. They were continued in mass matings thereafter. Fifteen to twenty pair matings were used in the experiment to distribute as evenly as possible any modifying genes still present in the stocks. Examination of the control data reveals no significant variation in mean bristle numbers during the course of the experiment.

The food used was a standardized corn-meal-molasses-agar mixture, with two drops of a yeast solution added to each culture. It was contained in half-pint milk bottles, about an inch in a bottle.

The incubators were regulated to a constancy of at least 0.5°C. The control incubator was at 25° and the experimental incubator at 41° to 42°C. Experimental cultures were kept with the control cultures excepting during the exposure period. Temperatures of the culture during exposure were read from a thermometer inserted in the middle of the food, where the larvae tend to congregate. It took approximately an hour for the temperature to rise to 40°C. when the cultures were placed in front of the bulbs heating the ends of the incubator. The temperature was approximately a degree lower in the middle of the incubator, in spite of the air currents set up by the constantly running fan. It was possible therefore to shift the cultures around and keep them at approximately 40°C. Three cultures were exposed at a time, and the temperature was read from one of them in each case. Tests showed that there was frequently a variation of approximately ±0.5°C. in the three cultures, with extremes at approximately 39.5° and 41.5°C. in the total number of experimental cultures. A large majority of the cultures, however, had temperatures ranging from 40° to 41°C., exclusive of the first hour. The temperature within the exposed cultures remained from 0.5° to 1° lower than the incubator temperature during the course of the exposure period. The thermometers showed no such differences *inter se* when placed side by side in the incubator. No difference in culture-incubator temperatures was observed in the cultures at 25°C.

Egg-laying periods were from four to six hours long in the experimental series, and up to twelve hours, with a few of one and two days, in the control series. The general plan was to secure two or three sets of experimental cultures during the day, and a set of control cultures during the night. Each set of parents was used for six to eight days, after having aged two days. Each experimental series contained cultures of from three to nine sets of parent flies.

The cultures were exposed at different periods of development for different periods of time. The developmental period of a culture was computed

TABLE 1

NUMBER OF BRISTLES PRESENT PER 100 SCUTE ♂♂

PERIOD OF DEVELOPMENT	SERIES	HOURS OF EXPOSURE, 40° TO 41°C. "a"	1/2	1	1 1/2	2	2 1/2	3	TOTAL "a"–3
0 to 10 hours (egg)	Exper.	21 ±1		20 ±2		20 ±2		20 ±15	21.0 ±1.0
	Control	10.5 ±0.6		12.0 ±0.7		12.0 ±0.7		12.0 ±0.7	12.0 ±0.7
20 to 30 hours (late egg and early first instar)	Exper.	10.3 ±0.4	16.9 ±0.7	15.5 ±0.7	11.0 ±0.6	7.6 ±0.5	29.9 ±0.9	29.5 ±0.9	17.8 ±0.2
	Control	9.2 ±0.4	10.2 ±0.3	10.8 ±0.3	10.6 ±0.3	9.7 ±0.3	11.2 ±0.3	10.6 ±0.3	10.2 ±0.3
48 to 58 hours (first half of second instar)	Exper.	15.0 ±0.8	15.9 ±0.7	14.9 ±0.7	19.4 ±0.8	26.6 ±0.8	19.7 ±0.8	19.9 ±0.9	18.8 ±0.3
	Control	9.9 ±0.4	9.2 ±0.3	9.4 ±0.3	9.6 ±0.4	9.2 ±0.3	9.4 ±0.3	9.6 ±0.4	9.4 ±0.3
72 to 82 hours (first quarter of third instar)	Exper.	19.5 ±0.8	20.0 ±1.0	19.2 ±1.1	17.2 ±1.0	22.0 ±1.1	18.4 ±1.0	18.2 ±0.9	19.4 ±0.4
	Control	9.2 ±0.3	9.7 ±0.4	10.2 ±0.4	9.8 ±0.4	9.8 ±0.4	9.7 ±0.4	9.5 ±0.4	9.7 ±0.4
96 to 106 hours (third quarter of third instar)	Exper.	6.2 ±0.6	10.6 ±0.9	9.5 ±0.8	14.8 ±1.0	13.9 ±0.9	13.2 ±0.9	7.7 ±0.7	10.6 ±0.3
	Control	9.2 ±0.3	9.2 ±0.3	9.2 ±0.3	9.6 ±0.4	9.4 ±0.3	9.3 ±0.3	10.6 ±0.4	9.3 ±0.3
120 to 130 hours (early pupal)	Exper.	11.9 ±0.9		13.8 ±0.9		12.5 ±0.8		15.1 ±1.0	13.3 ±0.4
	Control	9.4 ±0.3		9.4 ±0.3		9.2 ±0.3		9.4 ±0.3	9.3 ±0.3

from the time the parents were placed in the culture until the time the culture was removed from the exposure to high temperature. It was so regulated that in each case a large majority of the larvae were within a ten-hour developmental period through the exposure period. The developmental periods used were: 0 to 10 hours, 20 to 30 hours, 48 to 58 hours, 72 to 82 hours, 96 to 106 hours and 120 to 130 hours of development. In terms of development (Dobzhansky and Duncan[2]), these periods represent approximately: the egg, the late egg and early first instar, the first half of the second instar, the first quarter of the third instar, the third

quarter of the third instar and the early pupal developmental periods, respectively. A majority of the larvae had pupated at 120 hours. Occasional ones had pupated at 96 hours, and probably were ones developing from eggs which had started development before they were laid. Most of the larvae pupated within a ten-hour period.

Cultures were exposed for $^1/_2$, 1, $1^1/_2$, 2, $2^1/_2$ and 3 hours beyond the first hour in which the temperature was rising to 40°C. For convenience, the first hour will be referred to as "*a*." In the case of the egg and pupal periods, exposures were limited to "*a*," 1-, 2- and 3-hour groups; but in the other four periods the half-hour exposure groups were also carried through.

The bristle counts were made on males only, the F_1 of *sc* females and *v car* males. Each male was classified on both sides for the presence of the following bristles: anterior scutellar (*as*), posterior scutellar (*ps*), anterior notopleural (*anp*), post-vertical (*pv*), ocellar (*oc*) and anterior plus middle orbital (*or*). These were classed singly and in all the combinations in which they appeared. As reported by Child, it was found impossible to distinguish the anterior and middle orbital bristles. These have been grouped together.

Presentation of the Data.—Control cultures were run throughout the course of the experiment. These numbered 297 cultures and 56,340 flies of which 27,669 were males. It was found that when the cultures were grouped according to the number of flies hatching per culture, the mean numbers of bristles present per 100 *sc* males varied significantly. In general, the mean numbers of the head bristles—*or*, *oc* and *pv*—decrease with an increasing mean number of flies hatching per culture, especially in the groups with the smaller numbers of flies hatching per culture; and the mean numbers of thoracic bristles—*anp*, *as* and *ps*—increase with an increasing mean number of flies hatching per culture, especially in the groups with the larger numbers of flies hatching per culture. Because of this effect of the number of larvae developing together in a culture upon the mean bristle number in *sc* males, the experimental data are compared with control data which represent mean bristle numbers that would have been expected had these experimental series been raised entirely at 25°C. and hatched the same mean numbers of flies per culture.

A total of 65,741 flies (32,060 males) were classified from the 600 cultures of the six experimental series. Only those data pertaining to the *oc* bristles are presented here. The other data will be presented in a later paper.

The accompanying table shows the number of *oc* bristles present per 100 *sc* males in the experimental series and in the corresponding controls. The data are given for each group in an experimental series, and for the totals of all the groups in each series. The "probable error" is included in each case.

In four of the six experimental series—the egg, the first half of the second instar, the first quarter of the third instar and the early pupal periods—the

mean numbers of *oc* bristles exceed the mean numbers in the control data in every group, and the differences between control and experimental series are clearly significant in the totals. The effect of the exposure was markedly lethal in the egg stage (the eggs did not hatch), so that the number of males observed was not so large as in the other series.

In the series exposed during the late egg and early first instar period the increase in mean *oc* bristle number is not uniform. The $^1/_2$- and 1-hour groups show an increase, and the $2^1/_2$- and 3-hour groups show a very great increase. The difference in all the groups totaled together is also large and statistically significant. In the series exposed during the third quarter of the third instar the increase seems to be limited to the $1^1/_2$-, 2- and $2^1/_2$-hour groups. The mean of these three groups together is significantly above the controls, and can be considered to establish a temperature effect even though the total for all the groups of this series is not certainly significantly above the controls.

The other bristles considered likewise showed non-uniform increases or decreases during these last two developmental periods, and reasonably uniform changes in the other four periods. This suggests a possible fundamental difference between the periods at the beginning and near the ending of the larval developmental period, and those periods entirely within the egg, larval or pupal developmental periods.

Discussion and Conclusions.—These data indicate clearly that the scute-1 phenotype has a temperature-effective period at 40° to 41°C. that extends at least from the time the egg is laid through the early pupal developmental period, a relatively long period, just as Child's data indicate clearly that for temperatures ranging from 14° to 31°C. the temperature-effective period is limited to a relatively short period late in the third larval instar. Other workers, using temperatures similar to those used by Child, have found more or less similarly limited temperature-effective periods for other phenotypic characters of Drosophila, including the mean number of bristles present in the Dichaete pattern, the size of vestigial wings and the mean number of facets in Bar eye and its alleles and heterozygotes. (See Child's paper for references.) In the light of this study it is legitimate to question the established limits of the temperature-effective periods for these other characters and the significance of any theories of development based upon limited temperature-effective periods. It becomes clearly evident that all the possibilities of experimental temperature technique must be tried before any character can be said definitely to have a temperature-effective period that is limited to one part of the developmental period.

* The Rufus B. Kellogg Research Fellow of Amherst College, Amherst, Massachusetts.

[1] Child, G., *Genet.*, **20**, 109–155 (1935).

[2] Dobzhansky, Th., and Duncan, F. N., *Wilh. Roux Arch. Entw. Org.*, Bd. 130, Heft 1, 109–130 (1933).